To: FACDQ
From: Procedures Strike Team and the Technical Work Group
Subject: New Procedure
Date: 07/18/07

The attached procedure is the work product of the Single Laboratory Procedures strike team that met July 12-13th.

The procedure has been discussed on one technical workgroup call, at which two modifications were suggested in order to emphasize the measurement of precision and accuracy at the QL:

Modification 1
Change section 1.2.6

From:
If there are any precision and accuracy requirements for the QL, then determine the mean recovery and relative standard deviation of the QL spike results. If the precision and accuracy requirements are not met, then the spikes must be repeated at a higher concentration (resulting in a higher QL)

To:
Determine the mean recovery and relative standard deviation of the QL spike results. If precision and accuracy requirements exist and are not met, then the spikes must be repeated at a higher concentration (resulting in a higher QL)

Modification 2
Add a new section immediately after section 2.5
Precision and Accuracy Check: Determine the mean recovery and relative standard deviation of the QL spike results. If precision and accuracy requirements exist and are not met, then the QL and spiking level must be raised

The Technical Work Group will continue its review of the procedure and may have additional changes or modifications. The intent in sending this document to you now is that it is the product of the Procedure Strike Team that FACDQ members have requested to see. Recognise that before a final procedure is submitted to the FACDQ, there may be additional changes.

DQFAC Single Laboratory DL – QL Procedure (Version 2.1)
7/16/2007

SCOPE[1]

Procedures are provided by which an individual laboratory may derive accurate estimates of routine method sensitivity for most analytical procedures.
These procedures set the Detection Limit (DL) at the lowest result that can be reliably distinguished from a blank (specifically a false positive rate of $\leq$ 1% is targeted). This is functionally equivalent to the ISO/IUPAC term Critical Value, L_C. The DL is the normal censoring limit for analytical result reporting.

The Quantitation Limit (QL) is set at the level that meets specific criteria that are defined within this procedure. The lowest calibration standard (or low level calibration verification standard for tests with a single point initial calibration) must be at or below the QL. A false negative rate of $\leq$ 5% for a true concentration at the QL is targeted. Additional criteria for an acceptable QL may be set within specific analytical methods or in other regulatory documents (in particular, limits on precision and accuracy). If these are required then they must be satisfied by the results from samples spiked at or close to the QL, as well as the requirements in this procedure.
The QL is based on elements of the both the detection limit (Ld) and the quantitation limit (Lq) using international terminology.

This procedure is not applicable to analytical methods for which it is not feasible to create spiked samples at increasing levels of concentration. For example, it does not apply to measurements of temperature or pH.

In some cases it is not necessary to report results below the quantitation limit. In these cases the determination of the DL may be omitted and only those steps necessary to define the QL need to be followed. If the DL and the QL are both required then all steps in the procedure should be followed.

GENERAL REQUIREMENTS

This procedure should be followed for each method where a DL and QL need to be determined. In order to form reliable estimates of detection and quantitation limits, all steps in a method must be followed during the collection of blank and low level spiked sample data. A method is defined as the combination of steps that are performed on a sample. For example, preparation steps such as liquid/liquid extraction must be performed as well as analytical steps such as gas chromatography.

1. INITIAL STARTUP

1.1. If no historical data are available proceed to Section 1.1.1. If historical data demonstrate that 50% or more of method blanks for an analyte give a numerical

[1] The FACDQ may want to make a recommendation regarding when the WQBEL is at the QL or DL.

result, then estimate a DL based on blanks as described in and beginning with section 1.1.3. If less than 50% of the historical method blank results give a numeric result then skip to Section 1.2. Numeric result include positive, negative, and zero values.

1.1.1. Collect results for method blanks generated during routine operation of the method. The method blank must go through all preparation and analysis steps of the method. A minimum of seven numerical method blank results, each from a different preparation batch, is required in order to calculate an initial estimate of the method DL. The minimum number of blanks needs to be analyzed on each instrument used to report data. If more than seven blank results are available then they should be used. In general, the greater the number of results used to create the estimate, the more accurate it will be.

1.1.2. If less than 50% of the method blank results give a numeric result then skip to Section 1.2.

1.1.3. If it is necessary to initiate analysis immediately, an estimate of the DL may be made by analyzing seven blanks in less than seven batches. This short term DL must be replaced by a DL determined from method blanks, in a minimum of seven different batches as soon as data are available in order to capture sufficient temporal variability.

1.1.4. If multiple instruments are to be used for the same test, and will have the same reporting limit or QL, a minimum of seven method blank results must be used for each instrument and a DL calculated for each instrument. If the same DL or QL is reported for multiple instruments, the laboratory shall use the highest DL for the purposes of reporting data, assuming client needs are achieved.

1.1.5. Results associated with known errors that occurred during analysis should be discarded, or where appropriate, corrected. It is also acceptable to apply a statistically accepted outlier test, such as the removal of results more than two or three standard deviations from the mean. Results two standard deviations or less from the mean should not be removed. With the exception of known errors, this data rejection must be performed with caution, and no more than 5% of data may be rejected. Excessive rejection of data will result in a calculated DL lower than can be supported.

1.1.6. If not all of the blanks have numerical results, but over 50% do, set the value for those blanks that do not have numerical results to zero. Calculate the sample standard deviation of the method blank results.

$$s = \sqrt{\frac{\sum_{i=1}^{n}(X_i - \overline{X})^2}{n-1}}$$

Where:
n = the number of results used in the calculation
X_i = a result obtained from the analysis of a sample

$\overline{X}$ = the mean of the results

1.1.7. Calculate the DL: $DL = \overline{X} + s\,t_{(n-1,1-\alpha=0.99)}$

Where:

- $\overline{X}$ is the mean result from the method blanks
- $t_{(n-1,1-\alpha=0.99)}$ is the 99th percentile of a t distribution with n-1 degrees of freedom. Values for t are listed in Table 1.

Note: In the case that a negative value for $\overline{X}$ is obtained, substitute zero for $\overline{X}$ in the equation for calculation of the DL.

1.1.8. If 5% or more blank results (after outlier removal) are greater than the DL, raise the DL as follows:

- to the highest result if less than 20 method blanks are available.
- to the next to the highest result if 20-100 method blanks are available.
- to the level exceeded by 1% of the method blanks if there are more than 100.

Only a blank that meets method specified qualitative identification criteria (where applicable) should be given a numerical result.

1.2. This section determines the DL for methods with less than 50% of blanks giving numerical results and also determines the QL for all methods.

1.2.1. If less than 50% of method blanks give numerical results then the DL is estimated using low level spiked samples. These spiked samples are also used to define the QL for all analytical methods.

1.2.2. Select the spiking level. The spiking level must be at or below the level that the laboratory intends to use as their QL for reporting. If an estimate of the DL has been made using method blanks, then the spiking level must be at least two times that DL. The laboratory may use prior experience or consideration of the signal to noise to form this estimate. All qualitative identification criteria in the analytical method must be met for spikes at the QL; (for example, identification of qualifier ions, ion ratios, etc). Where it is necessary to achieve the lowest QL possible, follow the optional procedure described in Section 1.2.2.1.

1.2.2.1. Using the laboratory's knowledge of the method, analyze spikes of the analyte(s) in blanks. Start at a measurable concentration and reduce the spike concentrations successively in steps of approximately 3 (e.g., 100, 30, 10, 3, 1 etc) until:

- signal to noise ratio is less than 3, or
- qualitative identification criteria are lost, or
- signal is lost, or
- the value is less than twice the detection limit determined in Section 1.1

Use the lowest concentration at which all the applicable criteria are met.

1.2.3. Test the selected spiking level.

1.2.3.1. Analyze at least a single spiked blank at the intended quantitation limit through the entire analytical procedure.

1.2.3.2. If the analyte is not detected, either because it does not yield a signal, or the result falls below a detection limit determined in Section 1.1., or qualitative identification criteria defined in the method are not achieved, repeat the test at twice the concentration used in Section 1.2.3.1.

1.2.3.3. If multiple instruments are to be used to perform the same test and the same reporting limit or quantitation limit will be used, then the test of the QL estimate must be performed on each instrument, and the highest value from all the instruments is used as the estimate.

1.2.4. Once the appropriate spiking level (which will become the QL) is selected, analyze a minimum of seven replicates, divided among at least three different preparation batches, each spiked at this level. If it is necessary to initiate analysis immediately, an estimate of the DL and QL may be made by analyzing seven QL spikes in less than three batches. The short term DL and QL must be replaced by a DL and QL determined from QL spikes in a minimum of three different batches as soon as possible.

1.2.5. If the analyte is not detected in any one of the replicates, analyze a minimum of seven replicates divided between three different preparation batches at twice the concentration. This new concentration is the QL estimate. If multiple instruments are used to report the same QL, at least two replicates in separate batches must be analyzed on each instrument.

1.2.6. If there are any precision and accuracy requirements for the QL, then determine the mean recovery and relative standard deviation of the QL spike results. If the precision and accuracy requirements are not met, then the spikes must be repeated at a higher concentration (resulting in a higher QL).

1.2.7. Estimate the DL. If the DL has been estimated using method blanks according to Section 1, skip this section and continue to Section 1.2.8. If the DL has not been estimated using method blanks (i.e., less than 50% of method blanks had numerical results) then the DL is determined according to the following equation:

$$DL = s \times t_{(n-1,1-\alpha=0.99)}$$

- Where *s* is the standard deviation of the measured QL spike results.
- $t_{(n-1,1-\alpha=0.99)}$ is the 99th percentile of a *t* distribution with n-1 degrees of freedom. Values for *t* are listed in Table 1.

Note: The lowest achievable DL may be obtained by following the optional steps in Section 1.2.2.1.

1.2.8. If 5% or more blank results (after outlier removal) are greater than the DL, raise the DL as follows:

- to the highest result if less than 20 method blanks are available.
- to the next to the highest result if 20-100 method blanks are available.
- to the level exceeded by 1% of the method blanks if there are more than 100.

Only a blank that meets method specified qualitative identification criteria (where applicable) should be given a numerical result.

1.2.9. Estimate the Lowest Expected Result (LER) from spikes at the QL.

$$\text{LER} = \frac{\overline{X}_s * QL}{SL} - \left(s \times t_{(n-1,1-\alpha=0.95)}\right)$$

- Where *s* is defined in Section 1.2.7.
- Where $\overline{X}_s$ is the mean concentration result from the QL spikes.
- $t_{(n-1,1-\alpha=0.95)}$ is the 95th percentile of a *t* distribution with n-1 degrees of freedom. Values for *t* are listed in Table 1.
- *SL* is the spike level used for the QL spike sample.

1.2.10. Compare the LER to the DL. If the LER is less than the DL then the QL is raised according to the equation:

$$\text{QL}_{\text{new}} = \frac{[DL + s * t_{(1-\alpha=0.95;n-1)}] * QLold}{\overline{X}_s}$$

1.2.11. Do NOT adjust the spiking level for ongoing QL verification (see Section 2) unless the spiking level is outside the range of half to twice the new QL. If qualitative identification criteria are not met at the spiking level, increase the spiking by a factor of two.

2. ONGOING VERIFICATION

2.1. At least once every 12 months, or more frequently at the discretion of the QA manager, re-evaluate the DLs and QLs.

2.2. Continue to collect method blanks with each batch from which data were reported and QL spikes for every analyte[2] at a rate of at least four per twelve month period (in separate batches). If multiple instruments are to be used for reporting data with the same DL and QL, use at least two spikes per instrument per twelve month period.

2.2.1. Evaluate your DLs and QLs at least every year using all of the spikes available in a twelve month period using the procedures described in the Sections below. All method blanks and QL spikes collected within a twelve month period should be used for reassessing DLs and QLs, unless there is reason to believe that the DL or QL changed substantially at some point during that twelve month period. In that case the most recent data may be used for the reassessment, but not less than 20 method blanks and seven QL spikes per instrument. More than twelve months worth of data may be used if there is no reason to believe that the DLs and QLs have changed.

2.2.2. Optionally, recalculate the DL using the formulas in 1.1.7. or 1.2.7.

2.3. **Blank Check**: For all methods, check the blank results against the DL. If 5% or more blank results (after outlier removal) are greater than the DL, raise the DL as follows:

- to the highest result if less than 20 method blanks are available.
- to the next to the highest result if 20-100 method blanks are available.
- to the level exceeded by 1% of the method blanks if there are more than 100.

Only a blank that meets method specified qualitative identification criteria (where applicable) should be given a numerical result.

2.4. **Qualitative Identification Check**: At least 95% of the QL spiked data for each analyte must meet the qualitative identification criteria in the method. If 5% or more do not meet the qualitative criteria, then raise the QL and the spiking level to a level at which the qualitative identification criteria can be reliably met.

2.5. **Lowest Expected Result (LER) Check**: Estimate the lowest expected result (LER) from spikes at the QL. See Section 1.2.9.

2.5.1. Compare the LER to the DL. If the LER is less than the DL then the QL is raised according to the equation in Section 1.2.10.

[2] For multi component analytes a lab may want to use representative analytes to collect data for classes of compounds. For example: methods that include PCBs, toxaphene, and t chlordane a mixture of 1016 and 1260 PCB aroclors may be used to represent PCBs; toxaphene may be used to represent toxaphene and t chlordane.

2.5.2. Do NOT adjust the spiking level for ongoing QL verification (see Section 2) unless the spiking level is outside the range of half to twice the new QL. It is also necessary to adjust the spiking level if the spike results are not meeting the qualitative identification criteria in the method.

2.6. If the QL can be lowered by a factor of two ore more, without causing the LER to be below the DL, and qualitative identification can still be reliably maintained, then the QL, optionally, may be lowered. If the spiking level is then outside the range of half to twice the new QL, then the spiking concentration must be adjusted accordingly.

2.7. After verification, if the assessment process indicates that the DL or QL have increased by a factor of two or more, labs should investigate causes and take appropriate corrective action when necessary.

3. REPORTING DATA

3.1. The QL as described above is the lowest level for reporting quantitative results, but data may be reported down to the DL.
For example, if the QL is 2.0 and DL is 0.6 then results are reported as follows:

Instrument result	Reported Result
2.1	2.1
1.9	1.9J or DNQ
0.91	0.9J or 0.91J or DNQ
0.54	<0.6 or 0.6U
ND	<0.6 or 0.6U

"DNQ:" Detected, Not Quantified
"U": A flag indicating non-detect
"J": A flag indicating increased uncertainty in the results

4. MATRIX EFFECTS

4.1. Optionally, to demonstrate whether or not you can achieve your estimated DL and QL in a specific matrix:

1) analyze the unspiked matrix to demonstrate that the analyte is below the DL and,

2) analyze a QL spiked matrix to demonstrate that the QL criteria can be achieved.

This procedure as outlined below could be applied to various matrices providing an analyte free matrix could be obtained. The procedure outlined in 4.1 will not allow False Positives caused by a Matrix Effect to be distinguished from true positive results.

Table 1.

99^{th} and 95^{th} percentile *t* values for *n* replicates

n	$t_{(1-\alpha)}$=0.99	$t_{(1-\alpha)}$=0.95	n	$t_{(1-\alpha)}$=0.99	$t_{(1-\alpha)}$=0.95
7	3.143	1.943	54	2.399	1.674
8	2.998	1.895	55	2.397	1.674
9	2.896	1.860	56	2.396	1.673
10	2.821	1.833	57	2.395	1.673
11	2.764	1.812	58	2.394	1.672
12	2.718	1.796	59	2.392	1.672
13	2.681	1.782	60	2.391	1.671
14	2.650	1.771	61	2.390	1.671
15	2.624	1.761	62	2.389	1.670
16	2.602	1.753	63	2.388	1.670
17	2.583	1.746	64	2.387	1.669
18	2.567	1.740	65	2.386	1.669
19	2.552	1.734	66	2.385	1.669
20	2.539	1.729	67	2.384	1.668
21	2.528	1.725	68	2.383	1.668
22	2.518	1.721	69	2.382	1.668
23	2.508	1.717	70	2.382	1.667
24	2.500	1.714	71	2.381	1.667
25	2.492	1.711	72	2.380	1.667
26	2.485	1.708	73	2.379	1.666
27	2.479	1.706	74	2.379	1.666
28	2.473	1.703	75	2.378	1.666
29	2.467	1.701	76	2.377	1.665
30	2.462	1.699	77	2.376	1.665
31	2.457	1.697	78	2.376	1.665
32	2.453	1.696	79	2.375	1.665
33	2.449	1.694	80	2.374	1.664
34	2.445	1.692	81	2.374	1.664
35	2.441	1.691	82	2.373	1.664
36	2.438	1.690	83	2.373	1.664
37	2.434	1.688	84	2.372	1.663
38	2.431	1.687	85	2.372	1.663
39	2.429	1.686	86	2.371	1.663
40	2.426	1.685	87	2.370	1.663
41	2.423	1.684	88	2.370	1.663
42	2.421	1.683	89	2.369	1.662
43	2.418	1.682	90	2.369	1.662
44	2.416	1.681	91	2.368	1.662
45	2.414	1.680	92	2.368	1.662
46	2.412	1.679	93	2.368	1.662
47	2.410	1.679	94	2.367	1.661
48	2.408	1.678	95	2.367	1.661
49	2.407	1.677	96	2.366	1.661
50	2.405	1.677	97	2.366	1.661
51	2.403	1.676	98	2.365	1.661
52	2.402	1.675	99	2.365	1.661
53	2.400	1.675	100	2.365	1.660

If *n* >100 use values for *n*=100.